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Tea tannin components modify the induction of sister-chromatid exchanges and chromosome aberrations in mutagen-treated cultured mammalian cells and mice

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Summary

The modifying effects of tannin components extracted from green tea and black tea on mutagen-induced SCEs and chromosome aberrations were studied. These tannin components did not affect spontaneous SCEs and chromosome aberrations in cultured Chinese hamster cells. The frequency of SCEs and chromosome aberrations induced by mitomycin C (MMC) or UV was enhanced by the posttreatment with tea tannin components. When cells were posttreated with tea tannin components in the presence of metabolic enzymes of rat liver (S9 mix), the modifying effects on the induction of SCEs and chromosome aberrations by mutagens were complicated. MMC- and UV-induced SCEs and chromosome aberrations were suppressed by the posttreatment with tea tannin components at low concentrations (≤ 6.7 μg/ml) with S9 mix. At a high concentration of tea tannin components (20 μg/ml) with S9 mix, a co-mutagenic effect was observed. The modifying effects of tea tannin components were shown to occur in the G_1 phase of the cell cycle. In cells from a patient with xeroderma pigmentosum (XP) and a normal human embryo, MMC-induced SCEs were suppressed by the posttreatment with tea tannin components in the presence of S9 mix, and enhanced in the absence of S9 mix. On the other hand, tea tannin components modified SCE frequencies in UV-irradiated normal human cells but not in UV-irradiated XP cells. Our results suggested that tea tannin components themselves inhibited DNA-excision repair and resulted in a co-mutagenic effect, while in the presence of S9 mix metabolites of tea tannin components promoted DNA-excision repair activity and resulted in an antimutagenic effect. MMC-induced chromosome aberrations in mouse bone marrow cells were suppressed by the pretreatment with green tea and black tea tannin mixture.

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Abbreviations: EC, (−)-epicatechin; ECg, (−)-epicatechin gallate; EGC, (−)-epigallocatechin; EGCg, (−)-epigallocatechin gallate; TF, free theaflavin; TFdg, theaflavin digallate; TFmgA, theaflavin monogallate A; TFmgB, theaflavin monogallate B.

In the past decade a number of mutagenesis-suppressing factors have been identified using bacterial cells, cultured mammalian cells and rodents. These antimutagenic factors are classified into several types according to their modes of action: inactivation of mutagens chemically or enzymatically, inhibition of metabolic activation

of promutagens, scavenging of free radicals produced by mutagens, and modification of repair activity (Ramel et al., 1986; Waters et al., 1990). Tea contains several antimutagenic components such as caffeine (Rothwell, 1974; Kakunaga, 1975; Nomura, 1976), ascorbic acid (Guttenplan, 1977, 1978), tea tannin components (Shimoi et al., 1986) and tannic acid (Shimoi et al., 1985; Sasaki et al., 1988, 1989). Green tea tannin mixture has been shown to decrease the frequencies of SCEs, chromosome aberrations and gene mutations induced by aflatoxin B_1 and benzo[*a*]pyrene in V79 cells and rats (Ito et al., 1989; Wang et al., 1989). Since tannin treatment was done before or simultaneously with the addition of promutagens in their studies, they assumed that the antimutagenic effect of tea tannin mixture was due to either an inhibitory effect of metabolic activation of promutagens or inactivation of ultimate mutagens or free radicals.

In this study, we investigated the effects of components of green tea tannin and black tea tannin on the induction of SCEs and chromosome aberrations by direct-acting mutagens in cultured mammalian cells and mice. We show here the data suggesting that tea tannin components act as comutagens and their metabolites act as antimutagens both based on the inhibition and promotion of DNA-excision repair activity.

Materials and methods

Chemicals

Tea tannin mixtures and their components were provided by Dr. Yukihiko Hara, Research Laboratories, Mitsui-Norin Co. Ltd., Shizuoka (Japan). Green tea tannin mixture consists of 54% EGCg, 13% ECg, 18% EGC, and 6% EC. Black tea tannin mixture contains 5% TF, 18% TFmgA, 18% TFmgB, 20% TFdg, 12% EGCg, 10% ECg, and 5% EC. The structures of tannin components are presented in Fig. 1. Mitomycin C (MMC) and 5-bromodeoxyuridine (BrdUrd) were obtained from Kyowa Hakko Kogyo Ltd., Tokyo (Japan) and Sigma Chemical, Inc., St Louis, MO (U.S.A.), respectively. Colchicine, TN16 and dimethyl sulfoxide (DMSO) were purchased from Wako Pure Chemical Industries, Osaka (Japan).

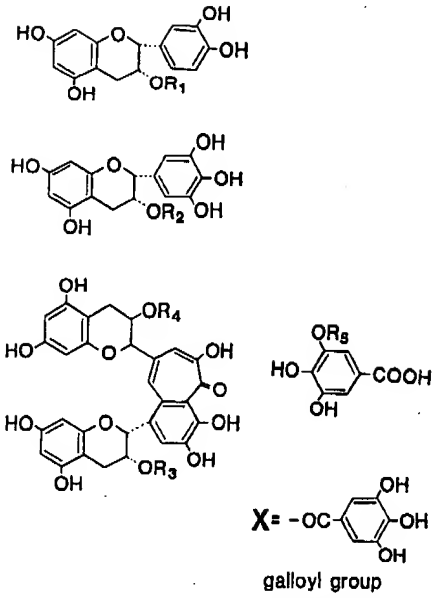

Fig. 1. Structures of tea tannin components. EC, $R_1 = H$; ECg, $R_1 = X$; EGC, $R_2 = H$; EGCg, $R_2 = X$; TF, $R_3 = R_4 = H$; TFdg, $R_3 = R_4 = X$; TFmgA, $R_3 = H$, $R_4 = X$; TFmgB, $R_3 = X$, $R_4 = H$; Tannic acid, $R_5 = X$.

All tea tannin components were dissolved in DMSO. The final concentration of DMSO in medium was 0.5%. MMC, colchicine and BrdUrd were dissolved in Hanks' balanced salt solution (pH 7.0). S9 fraction was prepared from the livers of male SD rats pretreated with polychlorinated biphenyl mixture (Aroclor 1254). The final concentration of S9 fraction in medium was 2.5%.

Cells and media

F7000 human cells from a normal human embryo and Chinese hamster ovary (CHO) K-1 cells were obtained from Flow Laboratories, Inc. and the American Type Culture Collection, respectively. XP3OS cells from a patient with xeroderma pigmentosum group A were obtained from Dr. Hiraku Takebe, Kyoto University (Japan). Human cells and CHO K-1 cells were grown in Dulbecco's MEM and Ham's F12 medium (Nissui Seiyaku Co. Ltd., Tokyo, Japan), respectively. The media contained 10% fetal bovine serum (FBS, HyClone Laboratories, Inc., U.S.A.), 50 IU/ml penicillin G, 50 μg/ml streptomycin sulfate and 2.5 μg/ml fungizon. All antibiotics were obtained from Flow Laboratories, Inc.

Modifying effects of tea tannin components

CHO K-1 cells and human cells were seeded at a density of $0.5-1.0 \times 10^6$ cells/100-mm dish and $0.5-1.0 \times 10^5$ cells/60-mm dish, respectively. Cells were treated with MMC for 1 h, and washed twice with Hanks' balanced salt solution. For X-ray or UV treatment, monolayers without medium were irradiated. Cells were then treated with each tea tannin component for 3 or 6 h with or without S9 mix. Thus the tannin component treatment was done after the mutagen treatment. For the analysis of SCEs, cells were cultured in the presence of BrdUrd at 20 μM for 2 cell cycles, and treated with colchicine at 50 μg/ml for 2 h before fixation. Preparations were processed using a modified Giemsa procedure (Sakanishi and Takayama, 1977) and harlequin-stained chromosomes in 50 metaphases per culture were analyzed for SCEs. For the assay of chromosome aberrations the same procedures were used except BrdUrd treatment. Preparations were stained with 2% Giemsa solution and 100 well-spread metaphases per culture were analyzed for chromosome aberrations. The number of chromatid breaks excluding gaps, chromatid inter- and intra-changes, and chromosome-type aberrations were counted. Data were statistically analyzed using Student's *t*-test.

SCE study in synchronized CHO cells

Following cultivation in the presence of BrdUrd for 1 cell cycle (21 h), the cells were treated for 20 h with 10 μM TN16, which has a colchicine-like effect (Sakata, 1981). Mitotic cells were collected by mitotic shake-off, seeded at a density of $0.5-1.0 \times 10^5$ cells/60-mm dish, treated with MMC at 1.0 μM for 1 h, and cultured in fresh medium containing BrdUrd. Pulse treatments with EGCg, TFmgB or TFdg with or without S9 mix were performed every 3 h following MMC treatment except for the first pulse treatment. The period of the first pulse treatment was 2 h. At the end of the pulse treatments with each tannin component, the cells were washed twice and recovered in medium with BrdUrd. The cells were treated with colchicine for 30 min and mitotic preparations were made 21 h after mitotic shake-off.

To determine the duration of cell-cycle stages (G_1, S, and G_2), the following experiment was carried out. M-arrested cells which had been treated with BrdUrd for 1 cell cycle were treat d with MMC and then pulse-treated with each tannin component as described above. At the end of the pulse treatments with each compound, the cells were washed twice and recovered in medium without BrdUrd. The replicational banding patterns on chromosomes were examined by a method described by Takayama et al. (1981).

G_1 holding study in CHO cells

In order to block cells in the G_1 phase (Tobey and Ley, 1970), CHO K-1 cells were incubated in an isoleucine-free medium supplemented with 10% dialyzed FBS (Gibco laboratories, Inc., U.S.A.) for 36 h. Cells irradiated with 15 or 20 J/m^2 UV were treated with EGCg, TGmgB or TFdg for 24 h without S9 mix, or for 6 h with S9 mix. The cells were washed twice and cultured in complete F12 medium with BrdUrd and were fixed 72 h later. Fifty metaphases per culture were analyzed for SCE frequencies in the second mitotic cells following G_1 block. For the assay of chromosome aberrations, treated cells were cultured for 24 h in complete F12 medium. Chromosome aberrations were analyzed in the first mitotic cells following the G_1 block.

Effects of tea tannin mixture in the mouse micronucleus test

Four male ddY mice (Japan SLC, Inc., Shizuoka, Japan) were randomly assigned to each treatment group. Tea tannin mixtures were suspended in distilled water. Mice received 2 mg/kg MMC intraperitoneally and were pre- or post-administered orally with green or black tea tannin mixtures at 500 mg/kg. The bone marrow cells were sampled 24 h after the injection of MMC. The assay of micronuclei was performed according to the method described by Schmid (1976). The incidence of polychromatic erythrocytes with micronuclei (MNPCEs) per 1000 polychromatic erythrocytes (PCEs) per mouse was determined. The mean frequencies of MMC-induced MNPCEs in mice with or without tea tannin mixture treatment were compared statistically using Student's *t*-test.

Results

Effects of tannin components on SCEs and chromosome aberrations in CHO cells

No tannin component induced SCEs and chromosome aberrations by itself or showed any effects on mutagen-untreated cells (data not shown). The modifying effects of posttreatments with tea tannin components on the frequency of MMC-induced SCEs and chromosome aberrations in CHO cells are shown in Table 1. The frequencies of MMC-induced SCEs and chromosome aberrations were increased by treatment with EGCg, ECg, TFmgA, TFmgB, or TFdg without S9 mix. On the other hand, when these tea tannin components were added to the culture medium at low concentrations (≤ 6.7 μg/ml) in the presence of S9 mix, significant decreases in the frequencies of MMC-induced SCEs and chromosome aberrations were observed. However, treatment with a high concentration (20 μg/ml) of tea tannin components with S9 mix caused an increase in the num-

TABLE 1

EFFECTS OF TEA TANNIN COMPONENTS ON SCEs AND CHROMOSOME ABERRATIONS INDUCED BY MMC

Tannin component (μg/ml)		SCEs/cell		Aberrations/cell	
		−S9 mix	+S9 mix	−S9 mix	+S9 mix
−	0	79.5	70.1	3.68	3.64
EGCg	2.0	79.8	60.1 **	3.33	0.93 **
	6.7	80.0	49.3 ***	5.56 $^{\#\#}$	1.16 ***
	20.0	107.8 $^{\#\#\#}$	94.3 $^{\#\#\#}$	8.30 $^{\#\#\#}$	7.91 $^{\#\#\#}$
ECg	2.0	87.3	66.2 *	3.36	1.22 ***
	6.7	88.6 $^{\#}$	55.0 ***	4.47 $^{\#}$	1.10 ***
	20.0	93.7 $^{\#\#\#}$	88.1 $^{\#\#\#}$	6.89 $^{\#\#\#}$	7.03 $^{\#\#\#}$
EGC	2.0	79.1	68.3	3.43	3.53
	6.7	81.3	69.1	3.30	3.84
	20.0	77.4	68.7	3.37	3.85
EC	2.0	79.7	70.1	3.47	3.50
	6.7	78.1	69.5	3.71	4.02
	20.0	76.3	67.2	3.57	3.85
TF	2.0	79.1	69.8	3.66	4.09
	6.7	78.8	67.1	3.59	3.81
	20.0	80.2	66.6	3.83	4.05
TFmgA	2.0	80.4	60.1 **	3.39	1.18 ***
	6.7	81.9	49.7 ***	5.30 $^{\#}$	1.32 ***
	20.0	100.2 $^{\#\#\#}$	84.3 $^{\#\#\#}$	5.57 $^{\#\#}$	5.23 $^{\#}$
TFmgB	2.0	82.5	58.2 **	3.61	1.12 ***
	6.7	95.0 $^{\#\#\#}$	55.8 ***	5.61 $^{\#\#}$	0.97 ***
	20.0	102.5 $^{\#\#\#}$	94.3 $^{\#\#\#}$	6.91 $^{\#\#\#}$	6.80 $^{\#\#\#}$
TFdg	2.0	79.9	54.4 ***	3.57	1.59 ***
	6.7	96.3 $^{\#\#\#}$	43.8 ***	6.01 $^{\#\#\#}$	1.24 ***
	20.0	97.2 $^{\#\#\#}$	90.2 $^{\#\#\#}$	7.80 $^{\#\#\#}$	7.89 $^{\#\#\#}$

CHO cells were treated with 20 μM MMC for 1 h and were posttreated with each tannin component for 6 h in the presence or absence of S9 mix.

Significant decrease: * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$.

Significant increase: $^{\#}$ $0.01 < P < 0.05$, $^{\#\#}$ $0.001 < P < 0.01$, $^{\#\#\#}$ $P < 0.001$.

Each value represents the mean of 3 experiments.

bers of SCEs and chromosome aberrations that was almost equal to the effect without S9 mix. EC, EGC and TF had no modifying effects at any concentrations. Similar effects of tea tannin components on UV-induced SCEs were observed (Fig. 2). UV-induced SCEs were suppressed by the posttreatment with EGCg, ECg, TFmgA, TFmgB, or TFdg at low concentrations ($\leqq 6.7$ μg/ml) in the presence of S9 mix. However, posttreatment with them in the absence of S9 mix or at a high concentration (20 μg/ml) in the presence of S9 mix increased SCEs. Tea tannin components with or without S9 mix did not show any cytotoxic effect on MMC- or UV-treated cells. On the other hand, X-ray-induced SCEs and chromosome aberrations were not affected by tea tannin components with and without S9 mix (data not shown).

The modifying effects of the posttreatment with tea tannin components on MMC-induced SCEs were investigated in synchronized cells. Synchronized cells were pulse-treated with EGCg, TFmgB or TFdg for 3 h at every cell-cycle stage. As shown in Fig. 3, SCEs were suppressed by tannin treatment with S9 mix in the G_1 phase. Tannin treatment without S9 mix in the G_1 phase increased SCEs. The effects of the posttreatment with EGCg,

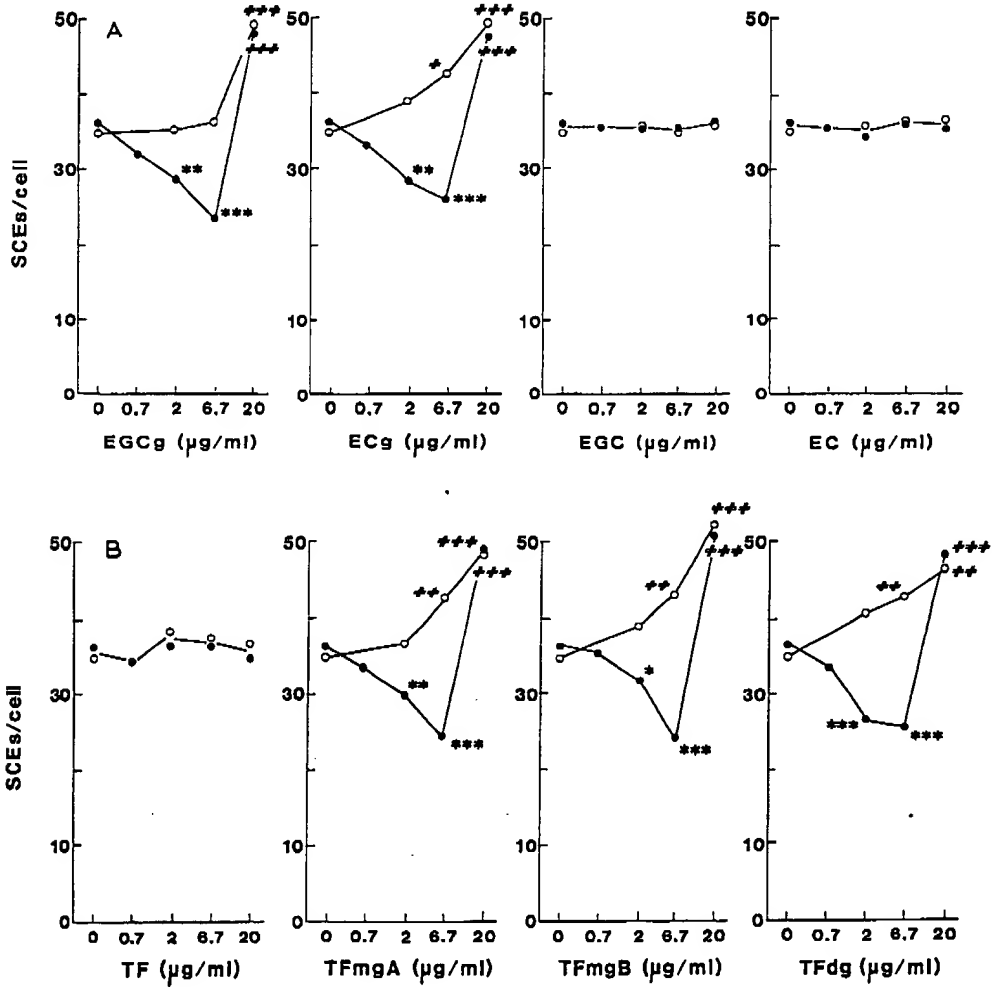

Fig. 2. Modifying effects of posttreatment with tea tannin components on UV-induced SCEs. Cells irradiated with 2.4 J/m^2 UV were posttreated with EGCg, TFmgB, and TFdg for 6 h with (●) or without (○) S9 mix. Significant decrease: * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Significant increase: # $0.01 < P < 0.05$, ## $0.001 < P < 0.01$, ### $P < 0.001$.

TABLE 2

EFFECTS OF TEA TANNIN COMPONENTS ON THE FREQUENCIES OF SCEs AND CHROMOSOME ABERRATIONS IN CELLS UNDER LIQUID HOLDING CONDITIONS

Tannin component (μg/ml)		SCEs/cell [a]			Aberrations/cell [b]		
	Holding time (h)	0	6 (+S9 mix)	24 (−S9 mix)	0	6 (+S9 mix)	24 (−S9 mix)
−		35.1	30.0	12.4	1.44	1.03	0.14
EGCg	0.7		28.1	nt		1.03	nt
	2.0		25.0 **	20.6 ###		0.80	0.78 ##
	6.7		22.2 ***	22.1 ###		0.62 *	0.87 ##
	20.0		nt	33.1 ###		nt	1.10 ###
TFmgB	0.7		30.2	nt		0.82	nt
	2.0		24.9 **	20.6 ###		0.58 *	0.22
	6.7		21.3 ***	23.4 ###		0.45 *	0.41 ##
	20.0		nt	25.4 ###		nt	0.69 ###
TFdg	0.7		29.3	nt		0.84	nt
	2.0		22.6 ***	19.8 ###		0.73	0.47 #
	6.7		21.9 ***	23.9 ###		0.59 *	0.67 ##
	20.0		nt	25.5 ###		nt	0.84 ###

[a] CHO cells held in G_1 phase were irradiated with 15 J/m^2 UV and were treated with tea tannin components in the presence or absence of S9 mix.

[b] CHO cells held in G_1 phase were irradiated with 20 J/m^2 UV and were treated with tea tannin components in the presence or absence of S9 mix.

nt: not tested.

Significant decrease: * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$.

Significant increase: # $0.01 < P < 0.05$, ## $0.001 < P < 0.01$, ### $P < 0.001$.

Each value represents the mean of 3 experiments.

TFmgB or TFdg on UV-induced SCEs and chromosome aberrations were further analyzed in G_1-phase holding cells. In the presence of S9 mix, the frequencies of UV-induced SCEs and chromosome aberrations were decreased gradually during liquid holding (Table 2). In the absence of S9 mix, a significant increase by tea tannin components was observed in G_1 holding cells. These results strongly suggested that tea tannin components and their metabolites modify the frequencies of SCEs and chromosome aberrations in the G_1 phase of the cell cycle.

Effects of tea tannin components on SCEs in human cells

The frequencies of SCEs induced by MMC were decreased by the posttreatment with EGCg, TFmgB or TFdg in the presence of S9 mix, while they were increased in the absence of S9 mix both in DNA-excision repair-defective XP cells and in normal human cells (Fig. 4). These tea tannin components with or without S9 mix also modified the frequency of UV-induced SCEs in normal cells, but not in XP cells.

Effect of tea tannin mixture on micronucleus formation in mice

Since tea tannin components showed an opposite effect in the presence and absence of S9 mix in cultured mammalian cells, it was interesting to know whether tea tannin mixture decreased or increased the frequency of chromosome aberrations in a whole mammalian system. Frequencies of MNPCEs induced by MMC in mouse bone marrow cells were reduced when tea tannin mixture was given orally to mice before the intraperitoneal injection of MMC. Pretreatment with green tea tannin mixture and black tea tannin mixture 6 h before the MMC injection decreased MNPCEs most markedly, by 83 and 78%, respectively (Table 3). Posttreatment with tea tannin mixture showed no suppressing effect on the fre-

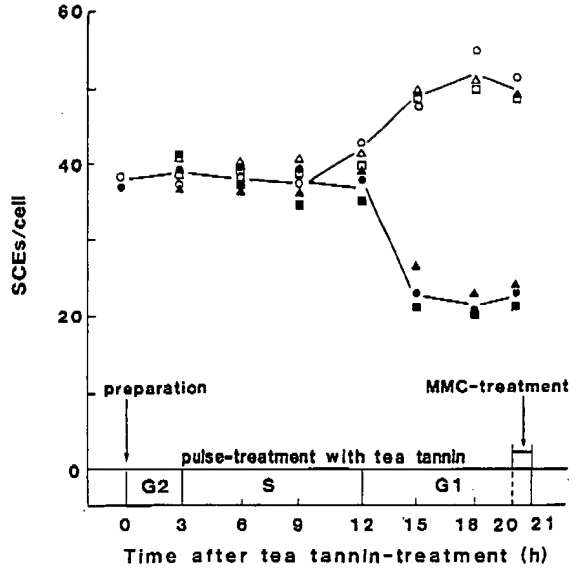

Fig. 3. Effects of tea tannin components at different cell stages on the frequencies of SCEs induced by MMC. Synchronized cells were treated with 1.0 μM MMC for 1 h and pulse-treated with 6.7 μg/ml EGCg (●), TFmgB (▲) or TFdg (■) for 3 h with S9 mix or with 20 μg/ml EGCg (○), TFmgB (△) or TFdg (□) for 3 h without S9 mix.

quencies of MMC-induced MNPCEs. No increase in MNPCE frequency by tea tannin mixture was observed at any concentrations investigated (data not shown).

Discussion

Antimutagenic effects of green tea tannin mixture, probably due to an inhibition of metabolic activation of promutagens, inactivation of ultimate mutagens or scavenging of free radicals, have been reported on mutagenesis induced by promutagens (Wang et al., 1989; Ito et al., 1989). In this study, we found that posttreatment with tea tannin components increased the frequency of MMC-induced SCEs and chromosome aberrations. A suppressing effect of tea tannin components was observed when cells were posttreated with ECg, EGCg, TFmgA, TFmgB and TFdg at low concentrations (≤ 6.7 μg/ml) in the presence of S9 mix. By contrast, at a high concentration (20 μg/ml) of tea tannin components with S9 mix, MMC-induced SCEs and chromosome aberrations

TABLE 3

EFFECTS OF PRE- AND POST-TREATMENT WITH TANNIN MIXTURE FROM GREEN TEA AND BLACK TEA AT DIFFERENT INTERVALS ON FREQUENCIES OF MMC-INDUCED MICRONUCLEI

Interval (h)	Green tea tannin mixture		Black tea tannin mixture	
	MNPCEs (%)	PCEs (%)	MNPCEs (%)	PCEs (%)
Pretreatment				
48	5.50±0.09	49.8±2.17	nt	nt
24	1.73±0.31 **	47.9±9.38	6.30±0.88	54.6±2.80
18	2.25±0.54 **	50.6±4.00	3.18±0.43 *	48.7±2.96
12	2.28±1.15 **	51.0±3.46	2.63±0.51 **	60.6±6.25
6	1.10±0.20 **	45.4±1.45	1.25±0.50 ***	53.9±1.02
Simultaneous treatment				
0	5.58±1.00	53.9±3.83	3.20±1.48 *	49.5±3.14
Posttreatment				
6	4.85±0.70	48.1±6.67	4.65±0.70	49.3±1.90
12	5.20±1.08	48.3±8.83	5.20±1.08	48.3±8.83
18	5.30±0.84	46.6±3.51	nt	nt
Control				
–	6.40±1.25	47.9±12.2	5.60±1.29	54.5±3.37

Mice received MMC at 2 mg/kg intraperitoneally and were pre- or post-administered orally with green or black tea tannin mixture at 500 mg/kg.
nt: not tested.
Each value represents the mean ± SD.
Significant decrease: * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$.

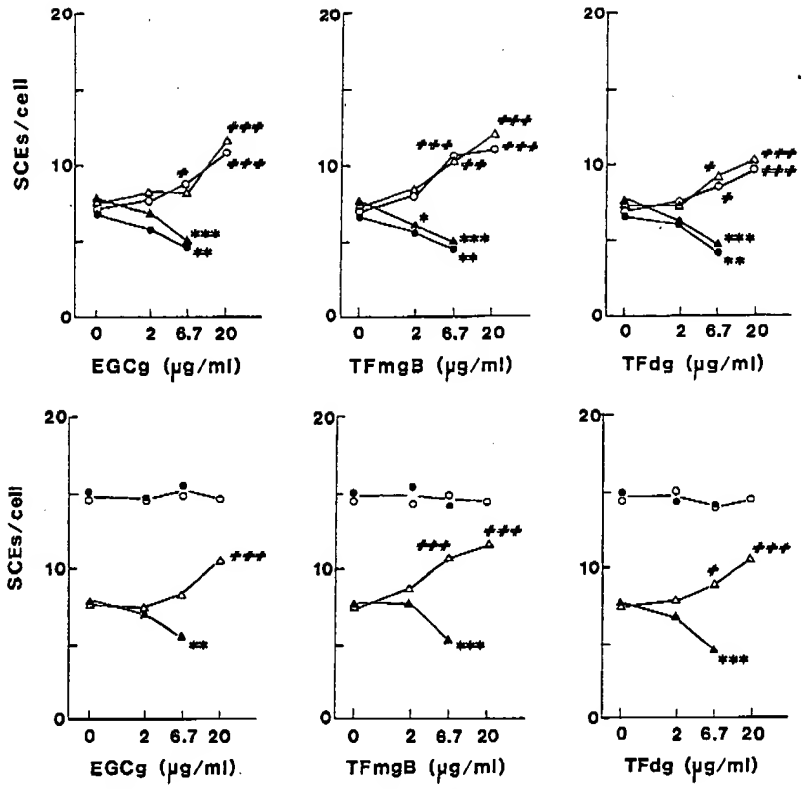

Fig. 4. Effects of tea tannin components on MMC- and UV-induced SCEs in normal human cells (top) and XP cells (bottom). Cells treated with 0.06 μM MMC for 1 h were posttreated with EGCg, TFmgB or TFdg for 3 h with (▲) or without (△) S9 mix. UV-irradiated cells at 1.0 J/m^2 were posttreated with EGCg, TFmgB or TFdg for 3 h with (●) or without (○) S9 mix. Significant decrease: * $0.01 < P < 0.05$, ** $0.001 < P < 0.01$, *** $P < 0.001$. Significant increase: # $0.01 < P < 0.05$, ## $0.001 < P < 0.01$, ### $P < 0.001$.

were enhanced. The suppressing effect of tea tannin components can be simply explained neither by inactivation of mutagens incorporated in cells by tannin components nor by inhibition of S9 activity, because MMC is a direct-acting mutagen and UV-induced SCEs were also suppressed. It is also unlikely that S9 enzymes inactivated the MMC which had been incorporated in the cells, because the frequency of MMC-induced SCEs and chromosome aberrations was almost the same in the presence and absence of S9 mix (Table 1). One possible explanation may be as follows. A small amount of tea tannin components ($\leq$ 6.7 μg/ml) would be metabolized by S9 mix and the metabolites would show antimutagenic effects. At a high concentration of tea tannin components (20 μg/ml) the activity of the S9 enzymes would be inhibited by the tannin components themselves, and, therefore, enough metabolites of tannin components would not be produced. This condition may be similar to that in the absence of S9 mix, where a co-mutagenic effect was observed.

Previously, we reported the enhancing effect of methyl sinapate and the suppressing effect of tannic acid on mutagen-induced SCEs and chromosome aberrations. These effects were considered to be due to the modification of the DNA-excision repair system based on the following findings: (1) posttreatment with methyl sinapate and tannic acid modified the frequencies of SCEs and chromosome aberrations induced by MMC and UV; (2) modifications were observed only in the G_1 phase of the cell cycle; (3) MMC-induced SCEs were modified in cultured cells both from a DNA-excision repair-deficient XP patient and from a normal human embryo, while UV-induced

SCEs were not affected in XP cells; (4) X-ray-induced SCEs and chromosome aberrations were not influenced by these compounds (Sasaki et al., 1988, 1989; Shimoi et al., 1989). The modes of modifying action of tannin components with and without metabolic enzymes resembled those of tannic acid and methyl sinapate, respectively. Therefore, we suppose tea tannin components themselves inhibit DNA-excision repair activity. On the other hand, metabolites of these compounds may promote DNA-excision repair activity and lead to the observed decrease in the frequencies of SCEs and chromosome aberrations. EGCg, ECg, TFmgA, TFmgB and TFdg have a galloyl moiety (Fig. 1). Since the metabolic process of tea tannin has not been well investigated, it is unclear whether tannic acid is produced. Identifying the metabolites that are responsible for the antimutagenic effect is an important problem to be solved. Another interesting question is why tea tannin mixture showed only a suppressing effect in vivo and why this suppressing effect was observed after pretreatment, but not posttreatment with tea tannins.

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